

Supplementary Information

The ankyrin repeat domain of Huntingtin interacting protein 14 contains a surface aromatic cage, a potential site for methyl-lysine binding

Tiyu Gao^{1,*}, Robert E. Collins^{1,*}, John R. Horton¹, Xing Zhang¹, Rongguang Zhang², Arunkumar Dhayalan³, Raluca Tamas³, Albert Jeltsch³, Xiaodong Cheng¹

¹Department of Biochemistry, Emory University School of Medicine, 1510 Clifton Road, Atlanta, GA 30322

²SBC-CAT, Advanced Photon Source, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439

³ Biochemistry Laboratory, School of Engineering and Science, Jacobs University Bremen, Campus Ring 1, 28759 Bremen, Germany

* These authors contribute equally to this work.

Correspondence should be addressed to:

Xiaodong Cheng

Phone: 404-727-8491

Fax: 404-727-3746

Email: xcheng@emory.edu

Submit to *Proteins: Structure, Function, and Bioinformatics* as a Structure Note

Key words: epigenetics; ankyrin repeats; methyllysine binding; Huntingtin interacting protein 14; X-ray crystallography

Supplementary Table 1. HIP14 substrates contain sequence(s) similar to histone H3 tail sequence

		4		9	
Histone H3		AR	TKQT	ARKST	
Huntington	MATLE	KLMK	AFESL	KSFQ	
		6	9	15	
hSNAP-25		EE	SK	DAGIRTL	
		FICPCNKL	KSS		
		RA	TK	MLGSG	
mGAD-65		NILLOQYVV	KSF		
		RS	TK	VIDFHYP	
		QTTLKYAI	KTG		
		RL	SK	VAPVIKA	
synaptotagmin I	AF	SK	LKEKFMN		
	MKDVKDLG	KTM			
	FE	TKV	HRKTL		
	RF	SK	HDIIGEF		
	NGKRLK	KKKT			

Supplementary Table 2. Methylated Huntingtin peptides used in the study

		6		9				15						
A	T	L	E	K	L	M	K	A	F	E	S	L	K	S
A	T	L	E	1	L	M	K	A	F	E	S	L	K	S
A	T	L	E	2	L	M	K	A	F	E	S	L	K	S
A	T	L	E	3	L	M	K	A	F	E	S	L	K	S
A	T	L	E	K	L	M	1	A	F	E	S	L	K	S
A	T	L	E	K	L	M	2	A	F	E	S	L	K	S
A	T	L	E	K	L	M	3	A	F	E	S	L	K	S
A	T	L	E	K	L	M	K	A	F	E	S	L	1	S
A	T	L	E	K	L	M	K	A	F	E	S	L	2	S
A	T	L	E	K	L	M	K	A	F	E	S	L	3	S

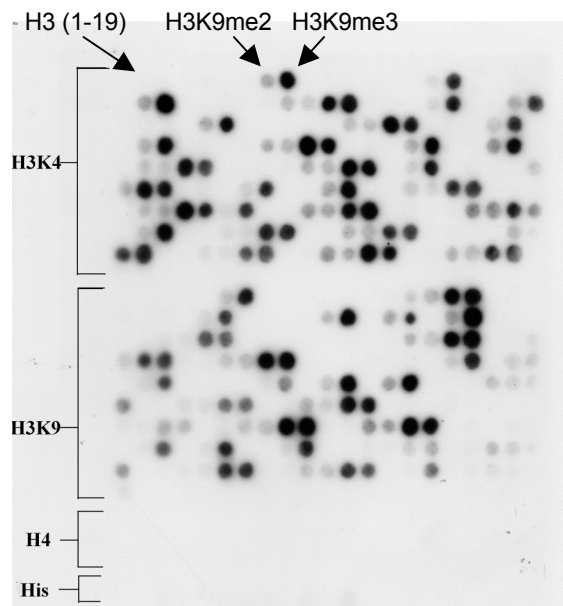
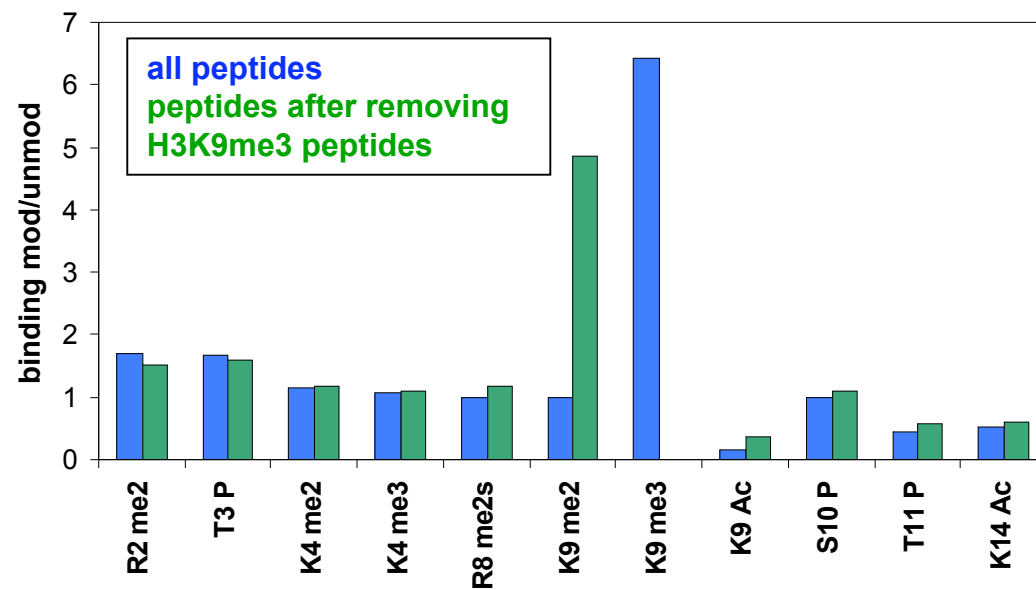
1: Kme1 - monomethyl lysine
2: Kme2 - dimethyl lysine
3: Kme3 - trimethyl lysine

Supplementary Figure 1. Binding of HP1 β chromodomain to peptide arrays

(A) Example of HP1 β binding to peptide arrays containing histone tail peptides containing different combinations of post translational modifications.

(B) Average of the binding intensities to all spots containing a particular modification divided by the binding to all spots not containing this modification. The data show a strong preference for binding to H3K9me3 (blue bars). After disregarding all H3K9me3 peptides, the analysis indicated that binding to H3K9me2 is the second next preference.

(C) Histogram of all peptide sequences present on the array. The peptides were sorted by binding intensity and the presence of some of the post translational modifications indicated.

A**B****C**